



Molecular Mechanisms of Sulfur Mustard

Vesicant-Induced Cell Death:


Early and late cell responses

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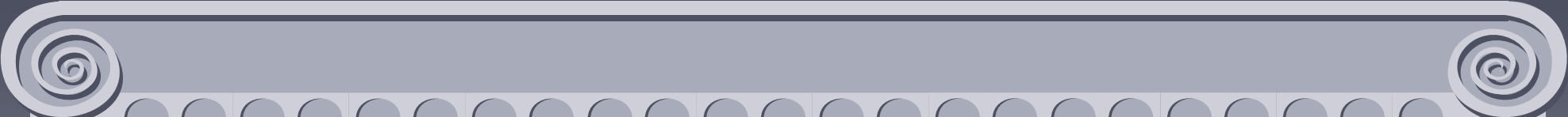
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Introduction



Sulphur mustard reacts with a wide range of biological molecules, including proteins and nucleic acids. It possess mutagenic, carcinogenic, cytotoxic, vesicating effects, and results in cell death.

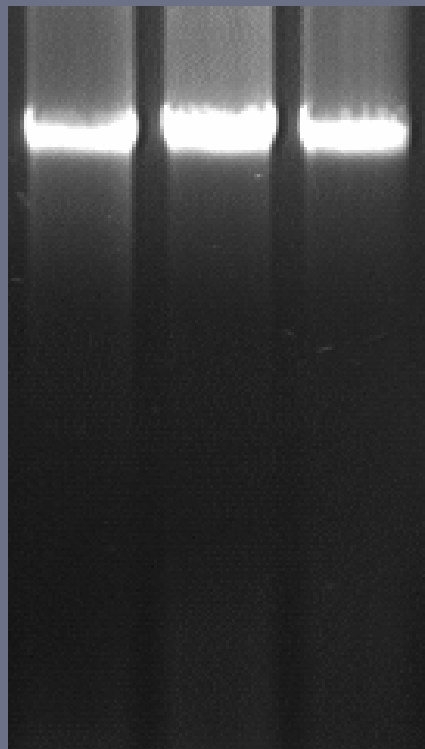
However, the biomedical mechanism of cell death induced by Sulphur mustard is not completely understood. To reveal this mechanism, we examined the specific genes involved in the regulation of cell survival and death pathway.



Experimental Results

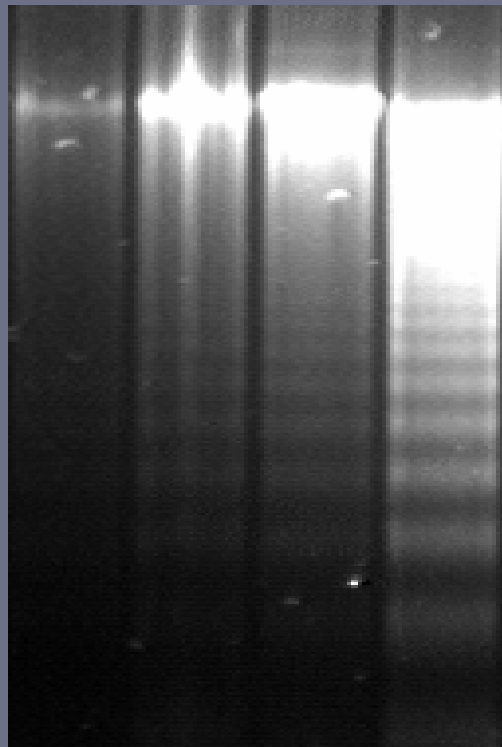
CEES Induced-Apoptosis

0 30 90 (min)

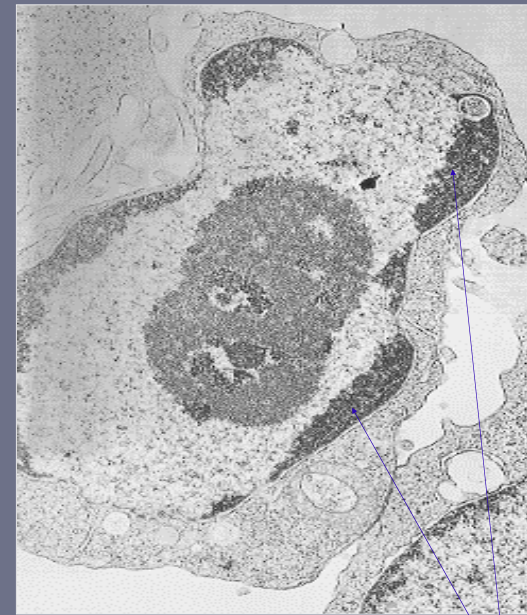


Early Stage

0 6 12 24 (h)

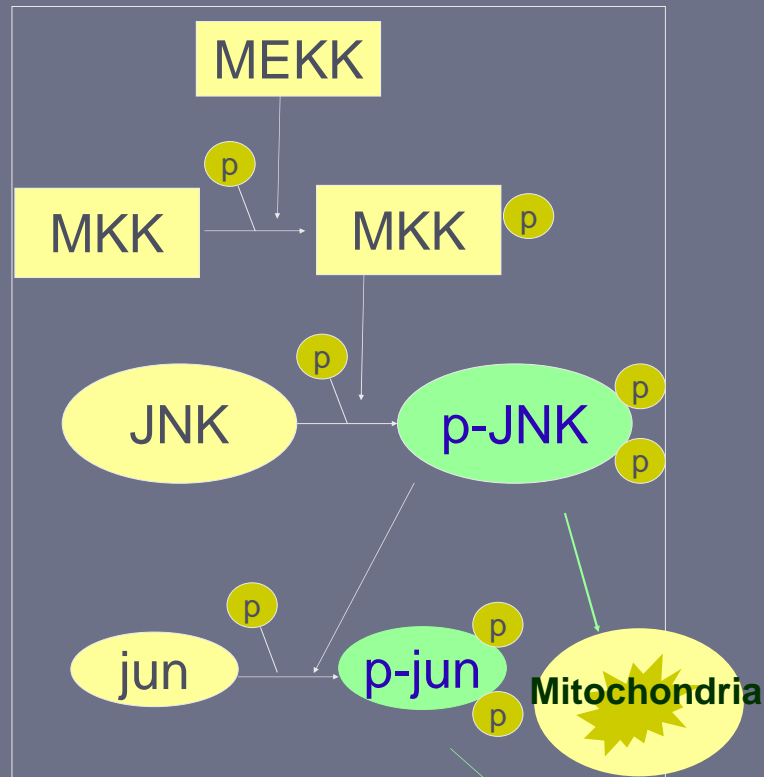


Late Stage

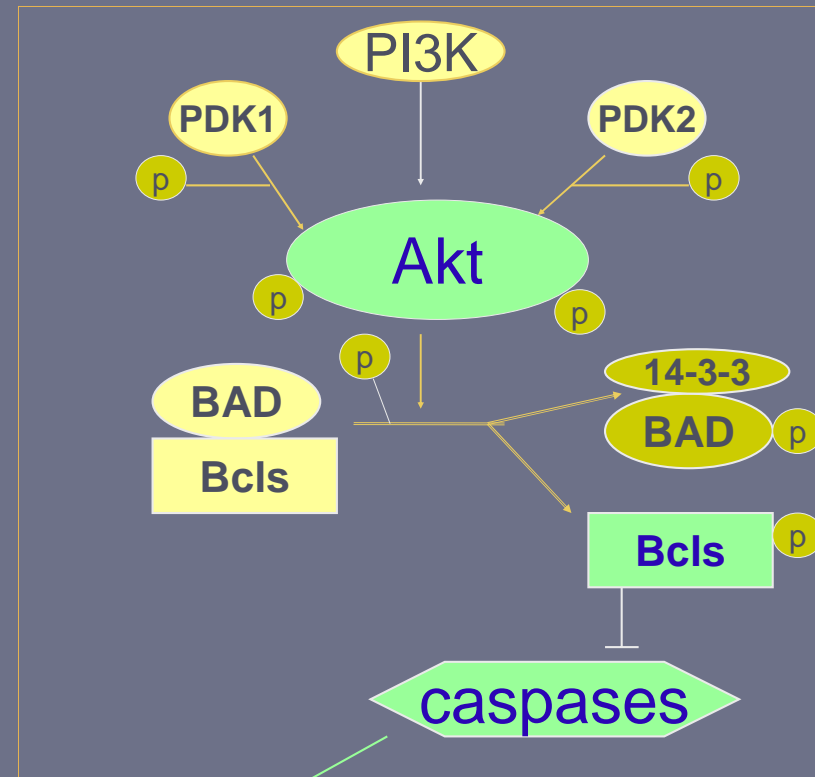


Perinuclear
margination
Of chromatin

Most Important Death Signal Transduction Pathway



Early Stage



Late Stage

Apoptosis 



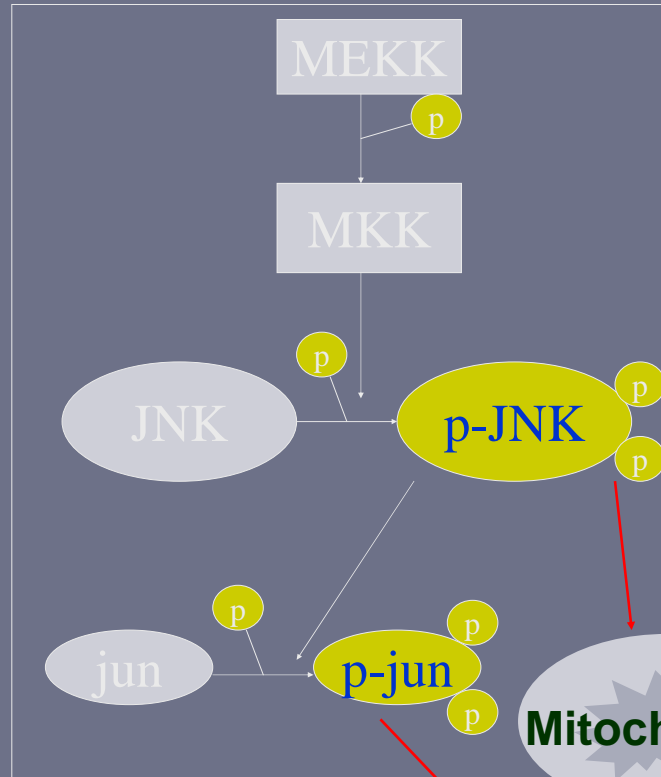
Cell Response to CEES

Damage in Early Stage

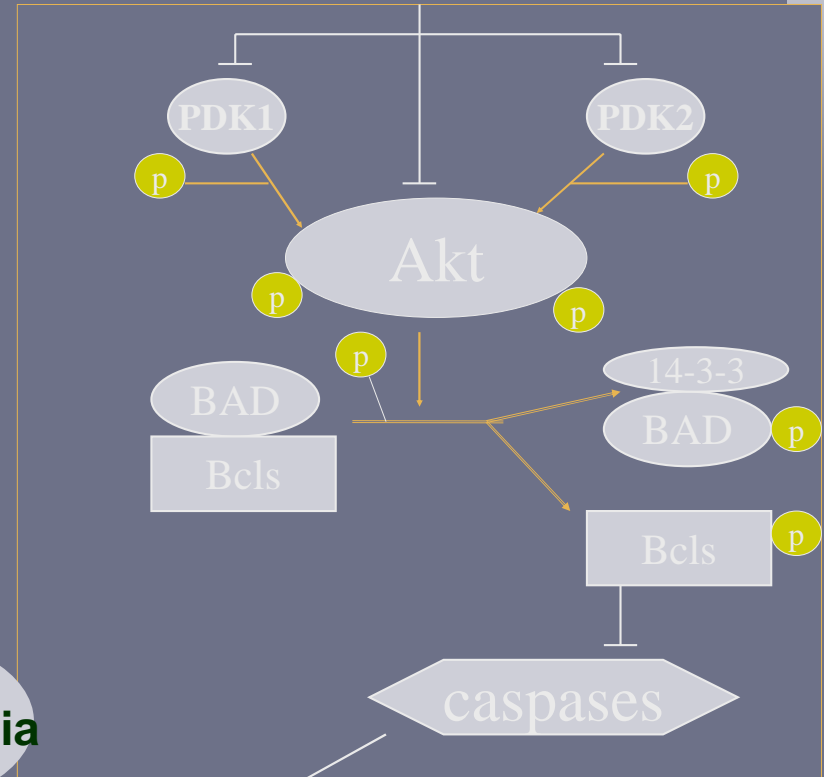
CEES

Attack

Cells



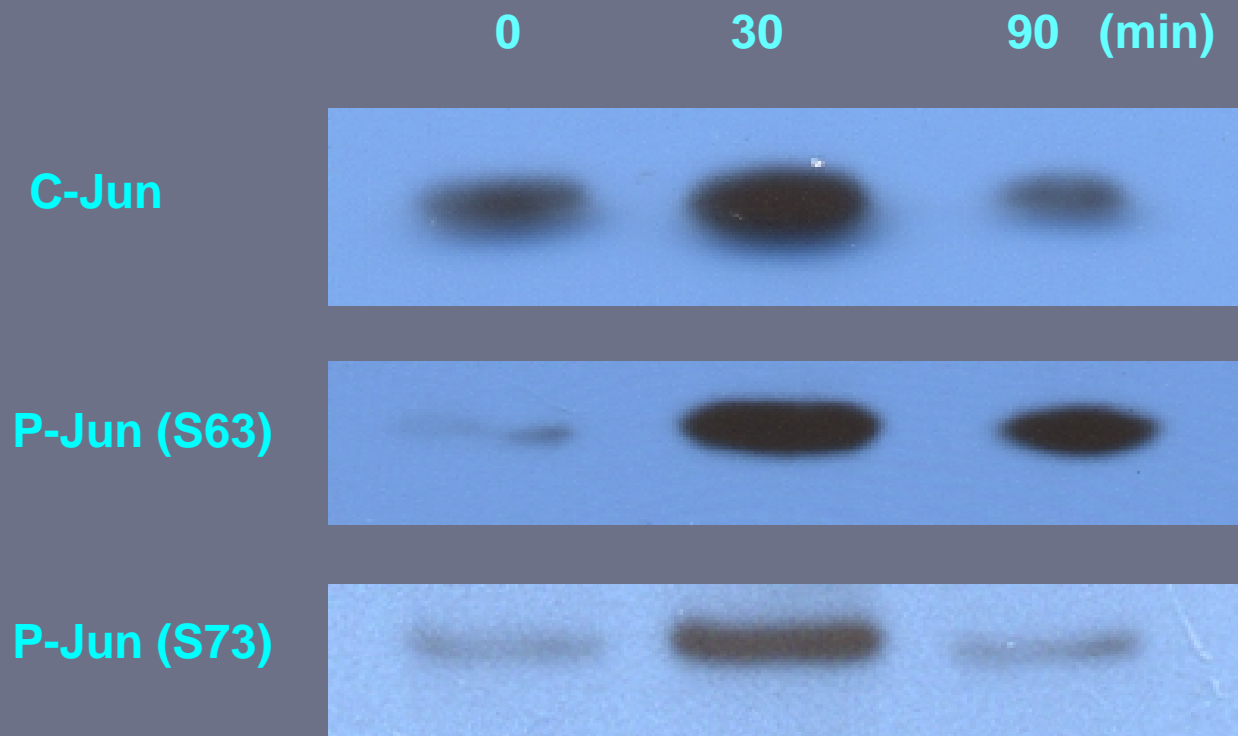
Early Stage



Late Stage

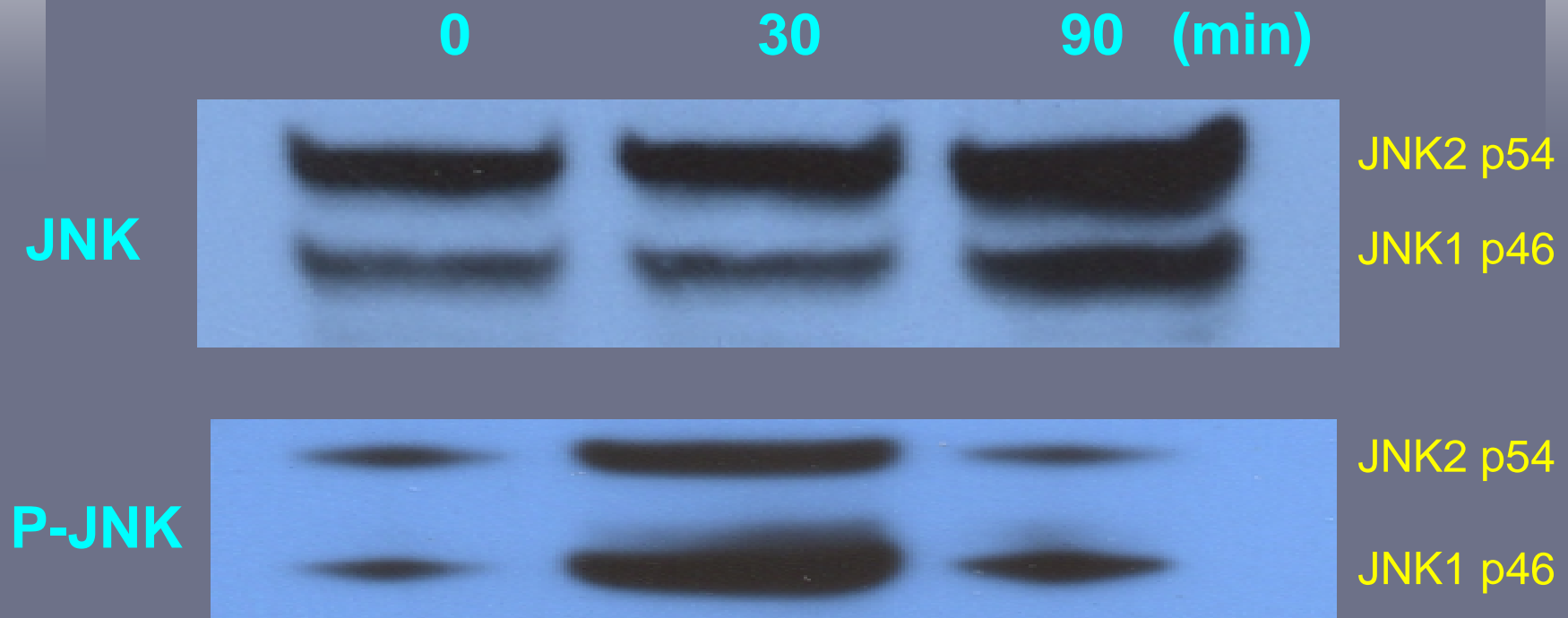
Apoptosis

CEES (200 μ M) Induced c-Jun and phosphorylation of Jun



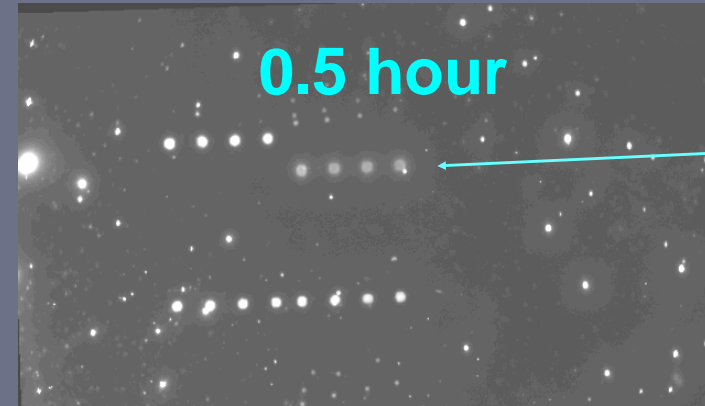
(Western Blotting)

CEES (200 μ M) Induced JNK and Phosphorylation of JNK



(Western Blotting)

Cytokines Induced in Early Stage of CEES (200 μ M) Damaged in Jurkat cells



•

[illegible]

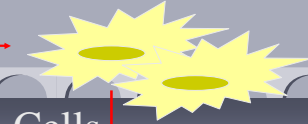


Cells Response to CEES

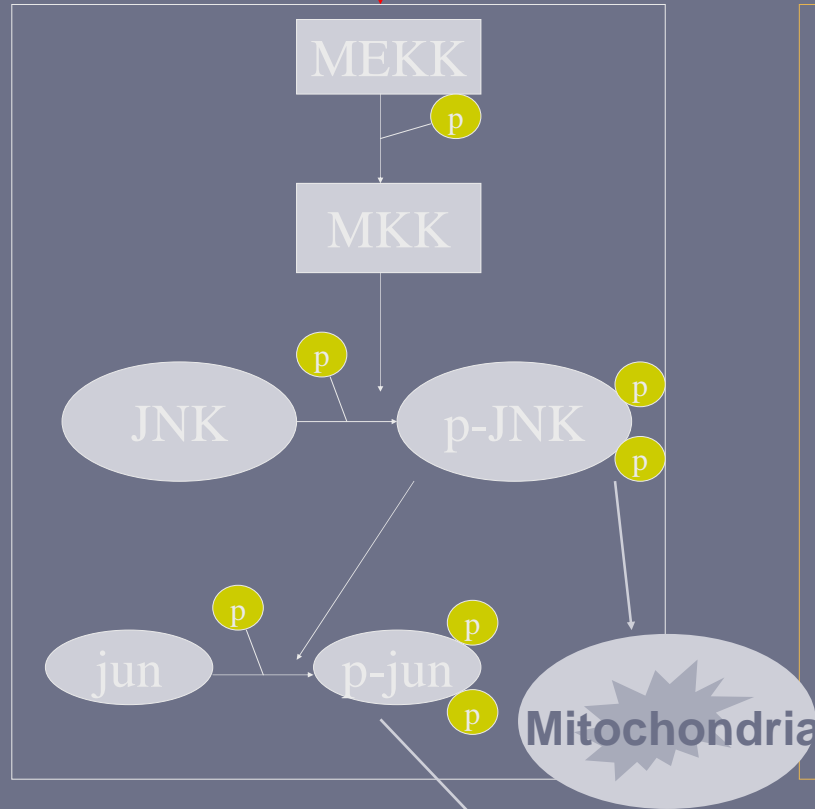
Damage in Late Stage

CEES

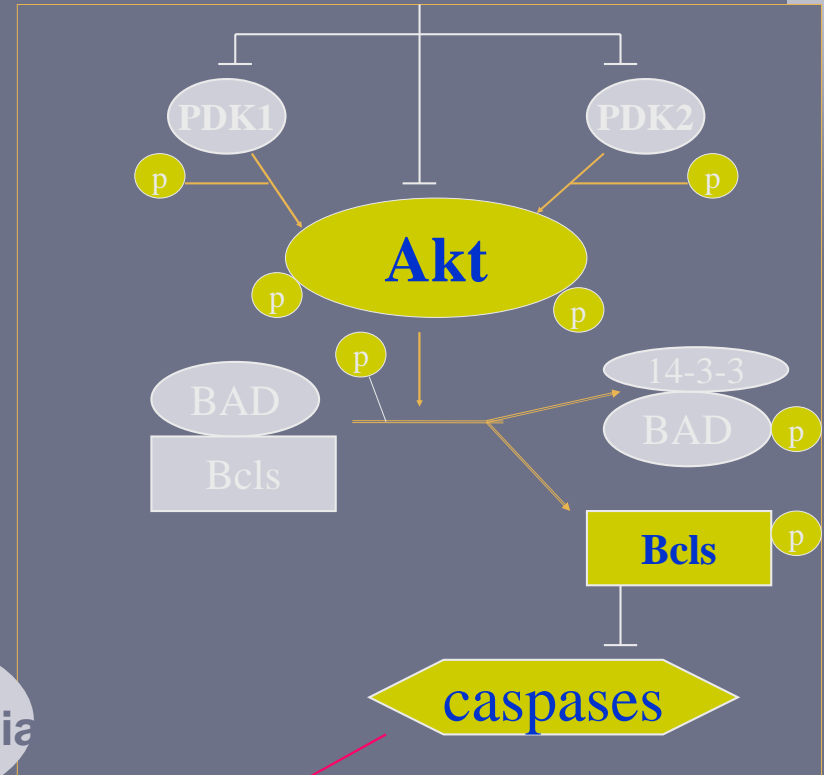
Attack



Cells



Early Stage

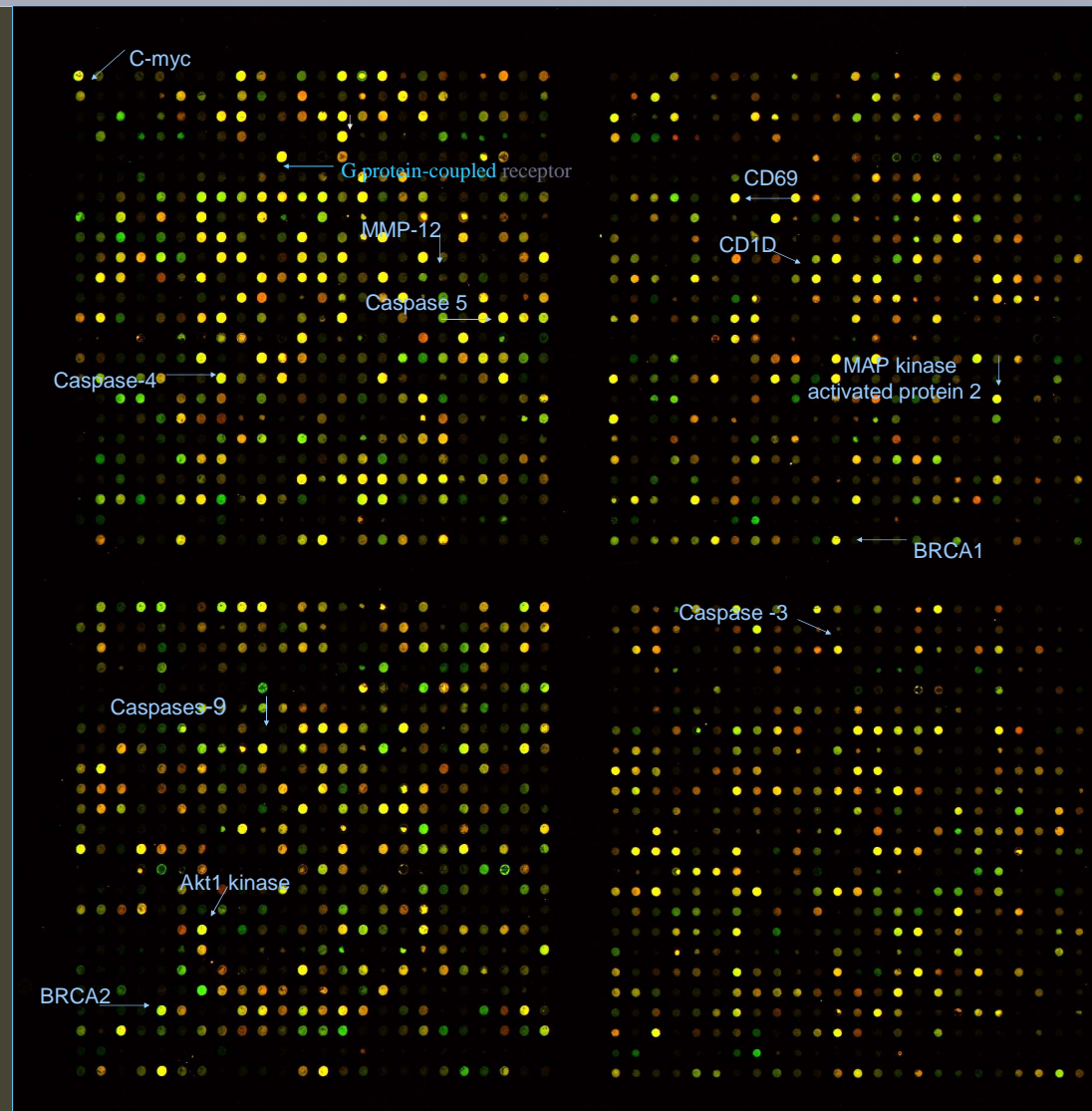


Late Stage

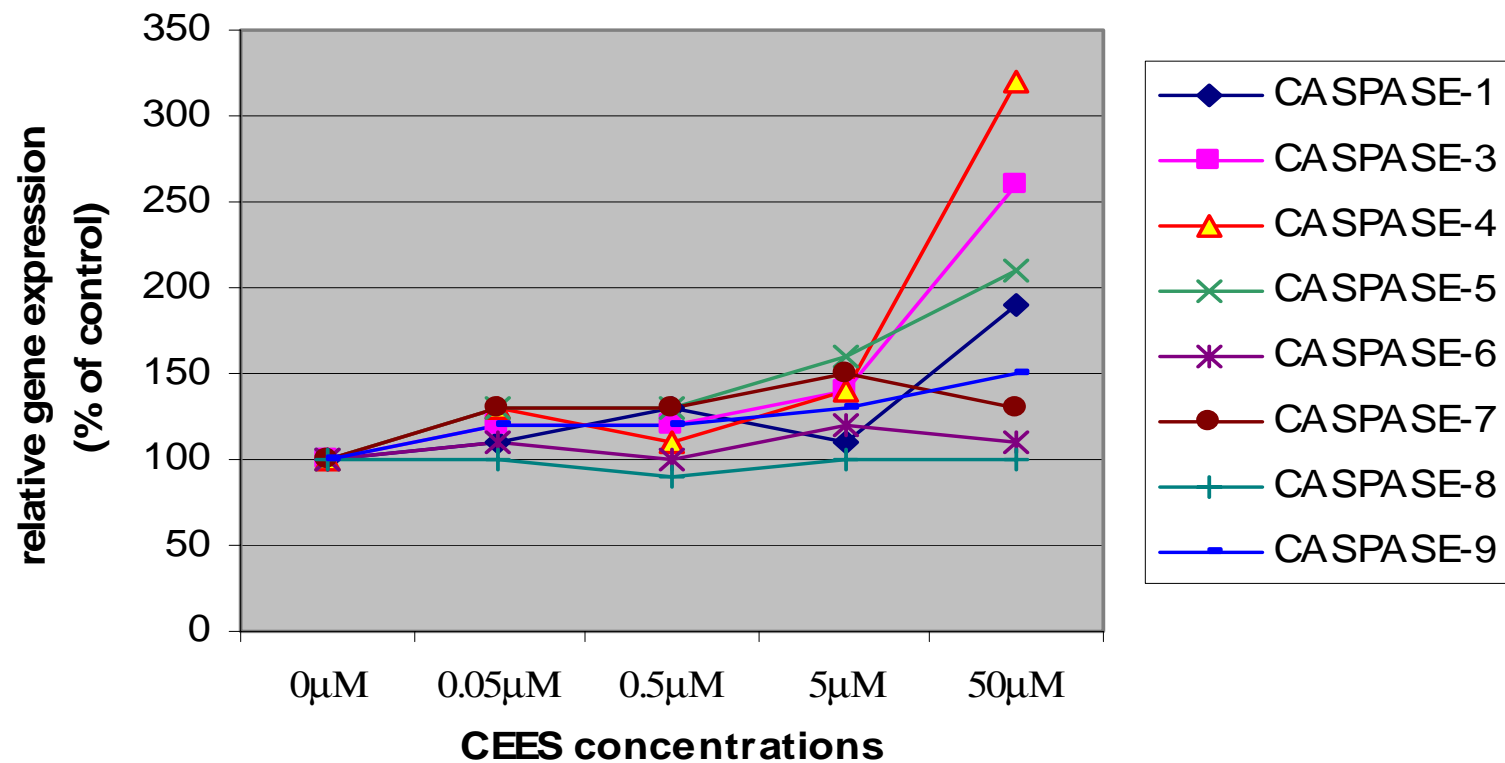


cDNA array

50 μ M CEES/24 hrs/Jurkat cells



Microarray reveals most caspases expression was induced by CEES

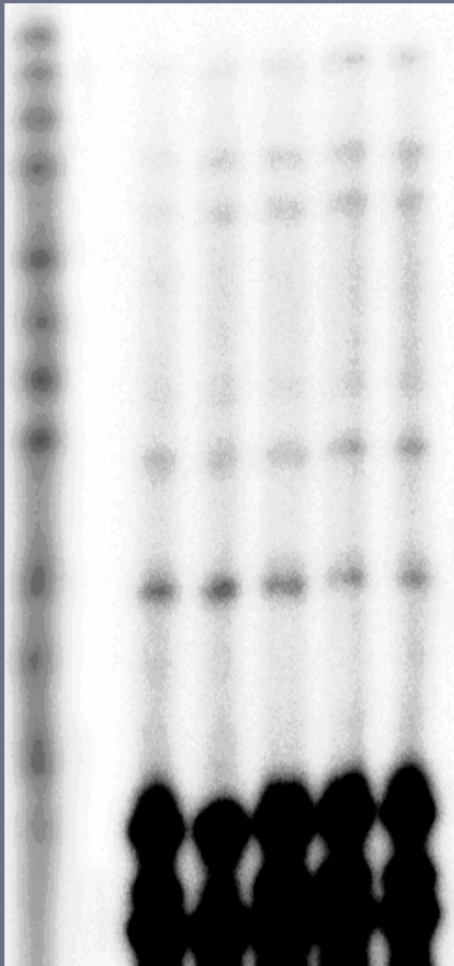


Array data was validated by RPA, RT-PCR, WB and activity assay

(A)

CEES[μ M] 0 0.05 0.5 5 50

435
390
350
312
256
238
210
192
156
141
124



Unprotected
(nt)

Caspase 8

Caspase 3

Caspase 6

Caspase 7

Caspase 1

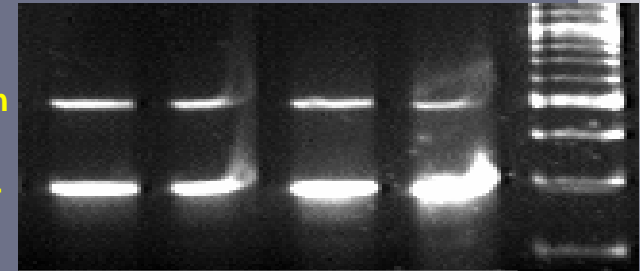
L32

GAPDH

(B)

β -actin

Caspase 4



(C)

CEES [μ M]

0 0.5 5 50

Caspase-3

— 32 kDa

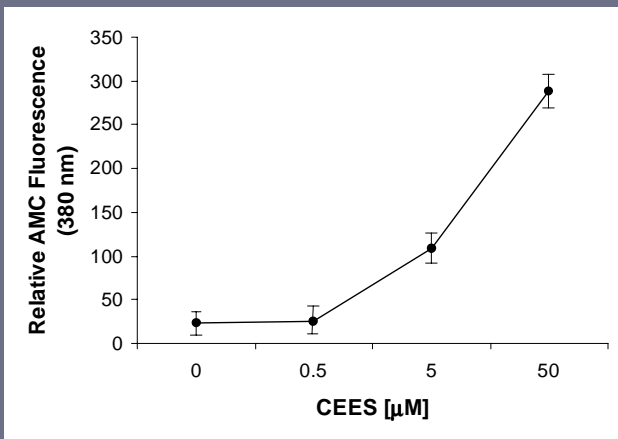
Caspase-4

— 45 kDa

— 40 kDa

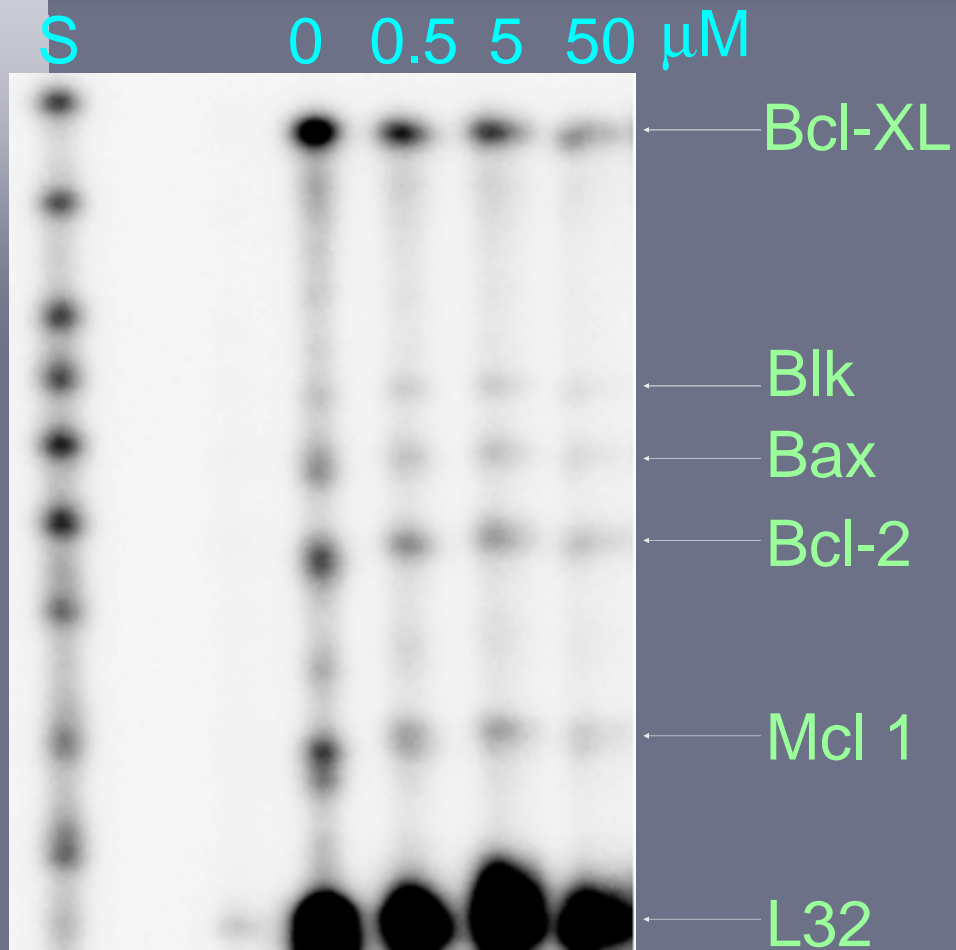
— 35 kDa

(D)

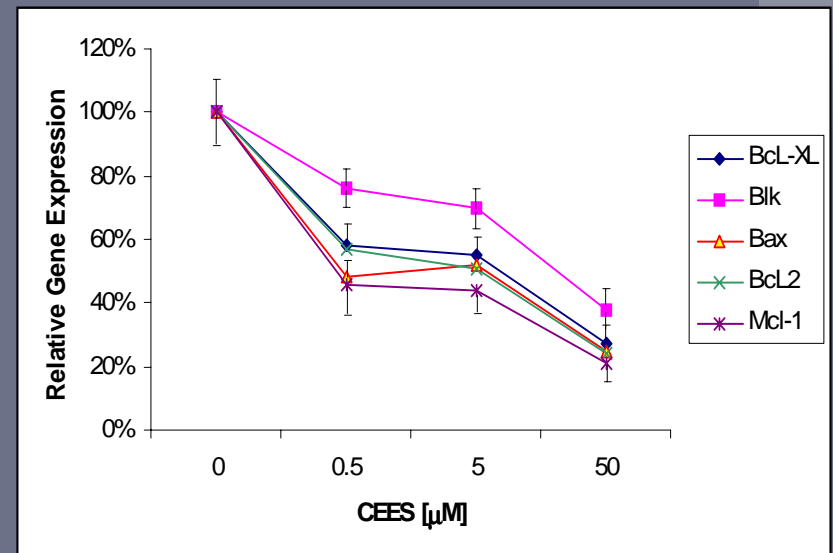


Caspase-3 enzyme Activity

CEES INHIBITED BCL FAMILY EXPRESSION



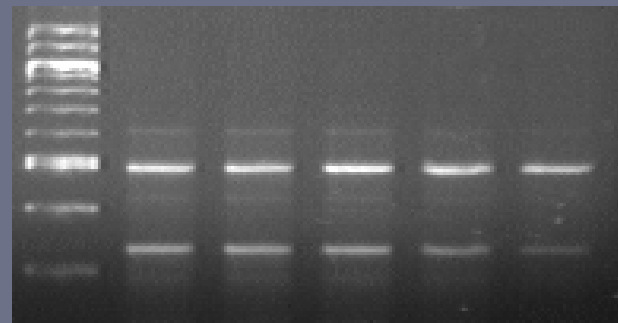
RNAse Protection



CEES Inhibited Akt/PKB expression

(A) RT-PCR

CEES[μ M] 0 0.05 0.5 5 50



β -actin

Akt

(B) Western Blotting

CEES [μ M]

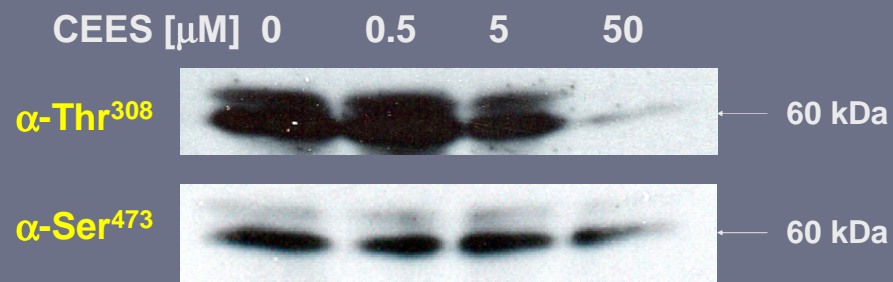
0 0.5 5 50



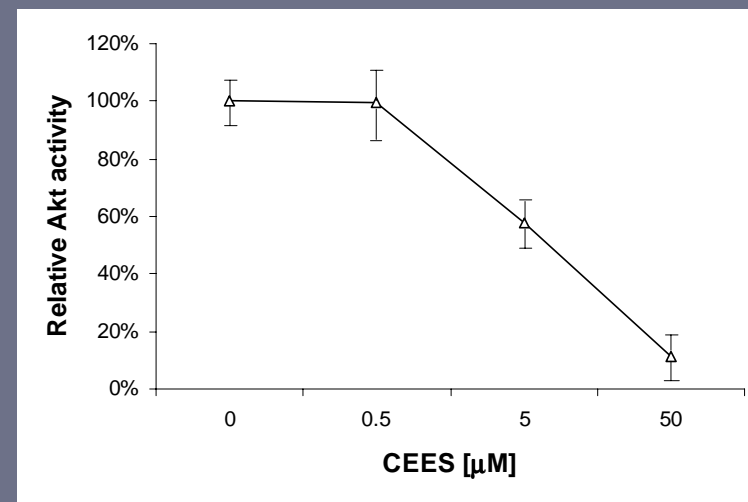
← 59 kDa

Phosphorylation and activities of Akt were affected by CEES

(A) Phosphorylation of Akt
(Western blotting)

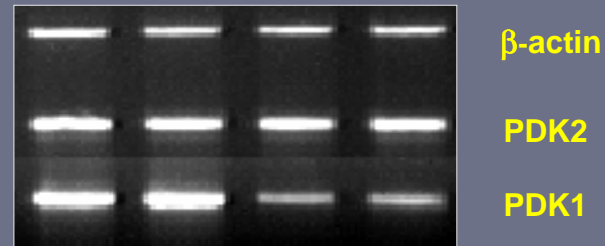


(B) Akt kinase activity assay

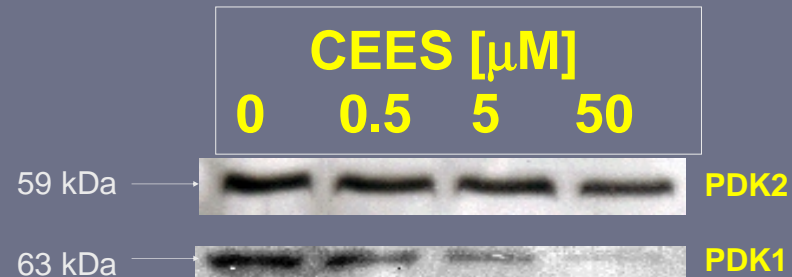


PDKs expression was affected by CEES

(A) RT-PCR



(B) Western Blotting



Cytokines Were Induced in Late Stage of CEEES Treatment (200 μ M) in Jurkat cells



IL-8

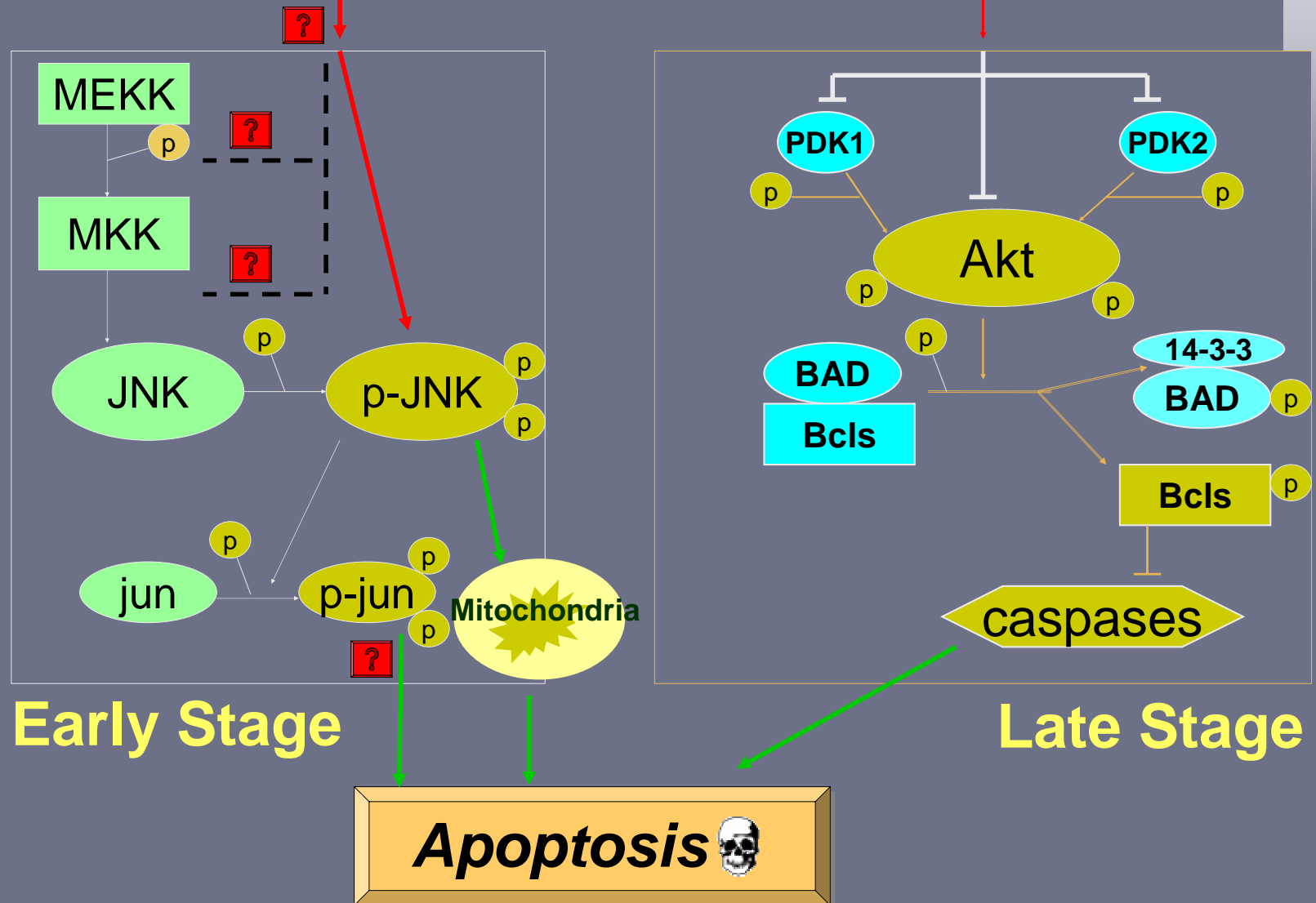
TNF- α


IL-1B

IL-10

Staining	Staining	Staining	Staining	Staining	Staining	Staining	Staining
TNF- α	TNF- α	TNF- α	TNF- α	IL-8	IL-8	IL-8	IL-8
IL-10	IL-10	IL-10	IL-10	PBS	PBS	PBS	PBS
PBS	PBS	PBS	PBS	IL-1B	IL-1B	IL-1B	IL-1B
IL-4 (Rat)	IL-4 (Rat)	IL-4 (Rat)	IL-4 (Rat)	IL-6	IL-6	IL-6	IL-6
Staining	Staining	Staining	Staining	Staining	Staining	Staining	Staining

(Protein array)





Conclusion

- Sulphur mustard causes cell death via apoptosis:
- In early stage, It induces JNK activity and then triggers apoptosis pathway.
- In late stage, sulphur mustard attacks the Akt pathway, by inhibiting Akt transcription, translation, and post-translation modification. Concomitantly, the anti-apoptotic genes, Bcl family, were down-regulated, in sharp contrast to the striking up-regulation of some death executioner genes, caspases 3, 8, 6 and 5.
- Sulphur mustard also induces some cytokines expression.
- Take together, sulphur mustard induces apoptosis by inhibiting the cellular survival factors which suppresses the expression of caspases.